

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application:

LISTING OF CLAIMS:

1. (currently amended): A process for obtaining yeast strains conserving stress resistance in the presence of fermentable sugars, comprising the following steps
a mutagenic treatment is carried out on ~~the~~ cells of a starting yeast strain,
the cells having undergone ~~the~~ said mutagenic treatment are cultured so as to obtain a stationary phase,
~~the~~ said cells in the stationary phase are incubated in the presence of at least one fermentable sugar selected from the group ~~comprising~~ consisting of glucose, maltose, and sucrose, ~~this wherein said fermentable sugar being is~~ present in a quantity such that the cells enter an active metabolic state ~~(fermentation and/or growth)~~ of fermentation and/or growth,
a population of said cells in the active metabolic state ~~are is~~ subjected to one or several stresses leading to a mortality rate of at least 99% with respect to the ~~starting~~ population,
the surviving cells are isolated and
~~those yeast strains of the surviving cells which respond to the following criteria which~~ characterize the that possess the following characteristics of a fil phenotype are selected;
 - ~~a growth of said selected yeast strains, evaluated by production or~~ determination of at least

one of the group consisting of biomass production over sugar in a given time, production yield of biomass over sugar in a given time ~~or by and~~ growth rate, which, under identical culture conditions, is at least equal to 80% of the growth of the starting yeast strain,

- ~~a~~-CO₂ release; or ~~a~~-metabolite production of said selected yeast strains, which, in identical conditions, is at least equal to 80% of the CO₂ release or metabolite production of the starting yeast strain,
- ~~a~~-stress resistance of said selected yeast strains, corresponding to a survival rate at least 2 times higher than the survival rate of the starting yeast strain, under identical phase conditions corresponding to ~~a~~-growth or active metabolism followed by a heat shock of at least 20 minutes at 52°C, or at least 1.5 times higher than the survival rate of the starting yeast strain, under identical conditions of said growth phase followed by freezing for a period of at least 24 hours at -20°C or at a lower temperature, and
- maintenance of ~~these~~ said growth, CO₂ release and stress resistance properties after repeated cultures of said selected yeast strains on non selective medium, so as to verify that the fil phenotype obtained by the mutation is perfectly stable and permanent thereby obtaining a yeast strain of the fil phenotype conserving stress resistance in the presence of fermentable sugars.

2. (currently amended): ~~A~~-The process according to claim 1, wherein it is checked that the selected yeast strains further present an alcohol assimilation, which, under identical conditions, is at least equal to 50% of the alcohol assimilation that of the starting yeast strain

and ~~that wherein~~ the selected yeast strains do not produce metabolites which give a bad smell or a bad or abnormal taste to breads.

3. (currently amended): ~~A~~ The process according to claim 1, wherein the starting yeast strain is an industrial strain.

4. (currently amended): ~~A~~ The process according to claim 3, ~~wherein an industrial fil~~
~~mutant carrying several mutations is obtained and wherein:~~

- ~~• the segregants issued from this industrial mutant are crossed with a~~
~~laboratory haploid strain to select the segregant issued from this industrial~~
~~mutant giving to the polyploids obtained with the laboratory strain an~~
~~improvement in the required properties;~~
- ~~• the segregants thus selected are crossed one with the other;~~
- ~~the polyploids obtained are selected according to the criteria of fil~~
~~phenotypes defined in claim 1~~ further comprising the steps of:
- obtaining segregants from a selected yeast strain carrying several
mutations,
- crossing said segregants with a laboratory haploid strain to obtain a first
family of polyploids, and selecting the segregants which by crossing with
the laboratory strains have produced polyploids of the first family with a
glucose consumption of cells after freezing, which, under identical
conditions, is equal to or higher than the glucose consumption of cells

after freezing of the starting yeast strain,

- crossing of the segregants thus selected with one another to obtain a second family of polyploids,
- selection of the polyploids of the second family with a glucose consumption of cells after freezing, which under identical conditions, is higher than the glucose consumption of cells after freezing of the selected yeast strain and that possess the following characteristics of a fil phenotype:

- growth of said polyploids of the second family, evaluated by determination of at least one of the group consisting of biomass production over sugar in a given time, production yield of biomass over sugar in a given time, and growth rate, which, under identical culture conditions, is at least equal to 80% of the growth of the starting yeast strain,
- CO₂ release or metabolite production of said polyploids of the second family, which in identical conditions, is at least equal to 80% of the CO₂ release or metabolite production of the starting yeast strain,
- stress resistance of said polyploids of the second family, corresponding to a survival rate at least 2 times higher than the survival rate of the starting yeast strain, under identical phase

conditions corresponding to growth or active metabolism
followed by a heat shock of at least 20 minutes at 52°C, or at least
1.5 times higher than the survival rate of the starting yeast strain,
under identical conditions of said growth phase followed by
freezing for a period of at least 24 hours at -20°C or at a lower
temperature, and

- maintenance of said growth, CO₂ release and stress resistance
properties after repeated cultures of said polyploids of the second
family on non selective medium,

thereby obtaining a yeast strain of the fil phenotype conserving stress resistance in the
presence of fermentable sugars.

5. (currently amended): ~~A~~ The process according to claim 1, wherein the ~~selected fil~~
~~strains~~ yeast strains of the fil phenotype have the property of conserving, in growth and/or
fermentation phase on fermentable sugars, at least 50% of their survival rate with respect
to the survival rate in stationary phase measured under the same conditions after a heat or
freeze shock.

6. (currently amended): ~~A~~ The process according to claim 1, wherein the cells obtained
after mutagenic treatment are introduced into pieces of dough subjected to at least 100
cycles of freezing/thawing after a first fermentation of the dough of 30 minutes at 30°C.

7. (currently amended): An industrial isolated yeast strain of the fil phenotype ~~having a survival rate, in growth phase on glucose, of at least 50% after heat treatment, the growth phase being defined as a cultivation of stationary cells on glucose for 10 minutes at 30°C after stationary phase~~obtainable by the process according to claim 1.

8. (canceled).

9. (currently amended): ~~A~~The industrial isolated yeast strain according to claim 7, belonging to *Saccharomyces cerevisia* species.

10. (currently amended): ~~A~~The industrial isolated yeast strain according to claim 7 having a survival rate, in growth phase on fermentable sugars, of at least 50% after a heat treatment of 20 minutes at 52°C, the growth phase being defined as a reculturing on glucose of 10 minutes at 30°C after stationary phase.

11. (canceled).

12. (currently amended): ~~A~~The industrial isolated yeast according to claim 7 whose stability to freezing in lumps of dough incubated 60 minutes at 30°C before freezing and containing 20 g of flour, 15 g of water, 1 g of sucrose, 0.405 g of NaCl, 0.06 g of (NH₄)₂SO₄ and 160 mg of dry matter of the considered strain, defined by the ratio between the release

of CO₂ at 30°C after 1 month or 30 days of conservation at -20°C and the release of CO₂ at 30°C after 1 day of conservation at -20°C, is at least equal to 80%.

13. (canceled).

14. (currently amended): ~~A~~ The industrial isolated yeast strain according to claim 57,
~~whose loss of released gas after~~ presenting a decrease in gassing power after drying of the
biomass of said industrial isolated yeast strain harvested in a phase close to exponential
growth phase, which is at most equal to 67% of the ~~loss of released gas after drying of~~
~~yeasts obtained using the corresponding starting strain~~ decrease in gassing power of the
starting yeast strain under identical conditions.

15. (currently amended): ~~Strain A strain~~ PVD1150 = M5fill deposited at Collection
Nationale de Cultures Microorganismes (C.N.C.M.) under the n° I-2031 and the n° I-2203.

16. (currently amended): ~~Strain A strain~~ KL1 = W303 fil2 deposited at C.N.C.M. under the
n° I-2032.

17. (currently amended): ~~Strain A strain~~ FD51 = HL816 fil300 deposited at C.N.C.M.
under the n° I-2033.

18. (currently amended): ~~Strain A strain~~ FDH16-22 = HL822 fil300 deposited at

C.N.C.M. under the n° I-2034.

19. (currently amended): ~~Strain~~ A strain AT25 = *S47 fil400* deposited at C.N.C.M. under the n° I-2035.

20. (currently amended): ~~Strain~~ A strain AT28 = *S47 fil500* deposited at C.N.C.M. under the n° I-2036.

21. (currently amended): ~~Strain~~ A strain AT251 deposited at C.N.C.M. under the n° I-2222.

22. (currently amended): ~~Strain~~ A strain AT252 deposited at C.N.C.M. under the n° I-2223.

23. (currently amended): ~~Strain~~ A strain AT254 deposited at C.N.C.M. under the n° I-2224.

24.–37. (canceled).

38. (currently amended): A dry baker's yeast obtained by culturing ~~a~~ an industrial isolated yeast strain according to claim 7.

39. (canceled).

40. (currently amended): A brewery yeast obtained by culturing ~~a~~an industrial isolated yeast strain according to claim 7.
41. (currently amended): A yeast intended for the production of alcohol obtained by culturing ~~a~~an industrial isolated yeast strain according to claim 7.
42. (currently amended): ~~A~~The process according to claim 1, wherein the yeast strains are of the *Saccharomyces cerevisiae* species.
43. (currently amended): ~~A~~The process according to claim 1, wherein the selected yeast strains present ~~a~~growth of said selected yeast strains, evaluated by determination of at least one of the group consisting of biomass production over sugar in a given time, production or production yield of biomass over sugar in a given time, and ~~or by~~ a growth rate, which, under identical culture conditions, is at least equal to 90% of the growth of the starting strain.
44. (currently amended): ~~A~~The process according to claim 1, wherein the selected yeast strains present ~~a~~CO₂ release; or a metabolite production of said selected yeast strains, which, in identical conditions, is at least equal to 90% of the starting yeast strain.
45. (currently amended): ~~A~~The process according to claim 1, wherein the selected yeast strains

present a stress resistance, corresponding to a survival rate at least 3 times higher than the survival rate of the starting yeast strain, under identical phase conditions corresponding to a growth or active metabolism followed by a heat shock of at least 20 minutes at 52°C, or at least 2 times higher than the survival rate of the starting yeast strain, under identical conditions of growth phase followed by freezing for a period of at least 24 hours at - 20°C or at a lower temperature.

46. (currently amended): ~~A-~~The process according to claim 1, wherein the selected yeast strains present a stress resistance, corresponding to a survival rate at least 5 times higher than the survival rate of the starting yeast strain, under identical phase conditions corresponding to a growth or active metabolism followed by a heat shock of at least 20 minutes at 52°C, or at least 3 times higher than the survival rate of the starting yeast strain, under identical conditions of growth phase followed by freezing for a period of at least 24 hours at - 20°C or at a lower temperature.

47. (currently amended): ~~A-~~The process according to claim 1, wherein the selected yeast strains present a stress resistance, corresponding to a survival rate at least 10 times higher than the survival rate of the starting yeast strain, under identical phase conditions corresponding to a growth or active metabolism followed by a heat shock of at least 20 minutes at 52°C, or at least 5 times higher than the survival rate of the starting yeast strain, under identical conditions of growth phase followed by freezing for a period of at least 24 hours at - 20°C or at a lower temperature.

48. (currently amended): ~~A-~~The process according to claim 1, wherein the ~~selected~~fil

~~strains~~obtained yeast strains of the fil phenotype have the property of conserving, in growth and/or fermentation phase on fermentable sugars, at least 60% of their survival rate with respect to the survival rate in stationary phase measured under the same conditions after a heat or freeze shock.

49. (currently amended): ~~A-The~~ process according to claim 1, wherein the ~~selected fil~~
~~strains~~obtained yeast strains of the fil phenotype have the property of conserving, in growth and/or fermentation phase on fermentable sugars, at least 70% of their survival rate with respect to the survival rate in stationary phase measured under the same conditions after a heat or freeze shock.

50. (currently amended): ~~A-The~~ process according to claim 1, wherein the ~~selected fil~~
~~strains~~obtained yeast strains of the fil phenotype have the property of conserving, in growth and/or fermentation phase on fermentable sugars, at least 80% of their survival rate with respect to the survival rate in stationary phase measured under the same conditions after a heat or freeze shock.

51. (currently amended): An industrial isolated yeast strain according to claim 7 belonging to the ~~Saccharomycetes~~ Saccharomyces genus

52. (currently amended): ~~A-An industrial isolated~~ yeast strain according to claim 7 having a

survival rate, in growth phase on fermentable sugars, of at least 60% after a heat treatment of 20 minutes at 52°C, the growth phase being defined as a reculturing on fermentable sugar of 10 minutes at 30°C after stationary phase.

53. (currently amended): ~~A~~ An industrial isolated yeast strain according to claim 7 having a survival rate, in growth phase on fermentable sugars, of at least 70% after a heat treatment of 20 minutes at 52°C, the growth phase being defined as a reculturing on fermentable sugar of 10 minutes at 30°C after stationary phase.

54. (currently amended): ~~A~~ An industrial isolated yeast strain according to claim 7 having a survival rate, in growth phase on fermentable sugars, of at least 75% after a heat treatment of 20 minutes at 52°C, the growth phase being defined as a reculturing on fermentable sugar of 10 minutes at 30°C after stationary phase.

55. (currently amended): An industrial isolated yeast according to claim 7 whose stability to freezing in lumps of dough incubated 60 minutes at 30°C before freezing and containing 20 g of flour 15 of water 1 g of sucrose, 0.405 of NaCl, 0.06 g of (NH₄)₂SO₄ and 160 mg of dry matter of the considered strain, defined by the ratio between the release of CO₂ at 30°C after 1 month or 30 days of conservation at -20°C, and the release of CO₂ at 30°C after 1 day of conservation at -20°C, is at least equal to 85%.

56. (currently amended): An industrial isolated yeast according to claim 7 whose stability to freezing in lumps of dough incubated 60 minutes at 30°C before freezing and containing 20 g of flour, 15 g of water, 1 g of sucrose, 0.405 g of NaCl, 0.06 g of (NH₄)₂SO₄ and 160 mg of dry matter of the ~~considered~~ considered strain, defined by the ratio between the release of CO₂ at 30 °C after 1 month or 30 days of conservation at -20°C and the release of CO₂ after 1 month or 30 days of conservation at -20°C and the release of CO₂ at 30°C after 1 day of conservation at -20°C, is at least equal to 90%.

57. (currently amended): An industrial isolated yeast strain ~~having~~ of the fil phenotype, obtainable by the process according to claim [[1]], presenting an alcohol assimilation, which, under identical conditions, is at least equal to 50% of that of the starting yeast strain and not producing metabolites which give a bad smell or a bad or abnormal taste to breads.

58. (currently amended): ~~A~~ An industrial isolated yeast strain according to claim 57, ~~whose loss of released gas~~ presenting a decrease in gassing power, after drying of ~~the~~ biomass harvested in a phase close to exponential growth phase, which, is at most equal to 50% of the ~~loss of released gas after drying of yeasts obtained using the corresponding starting strain~~ decrease in gassing power of the starting yeast strain under identical conditions.

59. (currently amended): A baker's yeast obtained by culturing ~~a~~ an industrial isolated yeast strain

according to claim 7.

60. (currently amended): An industrial isolated yeast strain of the fil phenotype obtainable by the process according to claim 1 and, presenting a stability to freezing in pieces of dough containing 20g of flour, 15g of water, 1 g of sucrose, 0.405 g of NaCl, 0.06g of $(\text{NH}_4)_2\text{SO}_4$ and an amount of the industrial yeast corresponding to 160mg of yeast dry matter, higher than 60%, said stability being defined as the ratio between the release of CO_2 at 30°C after 30 days of conservation at -20°C and the release of CO_2 at 30°C after 1 day of conservation at -20°C, whereby before freezing at -20°C, the pieces of dough are incubated at 30°C for 30 minutes.

61. (currently amended): An industrial isolated yeast strain of the fil phenotype obtainable by the process according to claim 1 and, presenting a stability to freezing in pieces of dough containing 20g of flour, 15g of water, 1 g of sucrose, 0.405 g of NaCl, 0.06 g of $(\text{NH}_4)_2\text{SO}_4$ and an amount of the industrial yeast corresponding to 160mg of yeast dry matter, higher than 80%, said stability being defined as the ratio between the release of CO_2 at 30°C after 30 days of conservation at -20°C and the release of CO_2 at 30°C after 1 day of conservation at -20°C, whereby before freezing at -20°C, the pieces of dough are incubated at 30°C for 30 minutes.

62. (new): An industrial isolated yeast strain according to claim 7 having a survival rate, in growth phase on fermentable sugars, of at least 50% after a heat treatment of 20 minutes at 52°C, the growth phase being defined as a reculturing of fermentable sugar of 10 minutes at 30°C after stationary phase.